

**A secreted protein of 15 kDa plays an important role in *Phytophthora palmivora*  
development and pathogenicity**

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## Supplementary figures

**Figure S1.** Amino acid sequence alignment of Ppal15kDa and its homologs in *Phytophthora* spp.

|                      |                |   |  |
|----------------------|----------------|---|--|
| <i>P. sojae</i>      | XP_009519869.1 | 1 | MSRLLQVLLVVMVALLASCNA--DIATKNQFTTATTNAAATTKALOKFFAEDSKONKKNQY  |
| <i>P. cinnamomi</i>  | PHYCI_93984T0  | 1 | MMRMLQVLLVIMVALLASCDAADTPKNHLLTISTTNAGATAKALOKFFADDAKONKKNQY   |
| <i>P. palmivora</i>  | POM75182.1     | 1 | --MRMIEVLVFLVAFASCHGAVAPTKNQLTISTKDSATAKALOKFFTEDAKKNKNQY      |
| <i>P. capsici</i>    | PHYCA_14775T0  | 1 | --MRMAQAFVLVILVALLSLWNCEAAATKNQLVISTTNGIATAKALQOFFVEDAKONNDKGF |
| <i>P. cinnamomi</i>  | PHYCI_81820T0  | 1 | MLHLRLRVLLVVLVLLANCEGTGTATKSOLTIATTNADATAKALOKFFVDDAKONKKNQY   |
| <i>P. megakarya</i>  | OWZ12090.1     | 1 | ---MFEVPLVFLVLLVLSCHGTITPTKNQLTISTRNSTATAKALOKFFTDKONKDSGF     |
| <i>P. parasitica</i> | XP_008909746.1 | 1 | MRVLFQALLVLLVALLVSCNEAANPTKHQLTITVTNSTATAKALOKFFNDKONKESNGF    |
| <i>P. cactorum</i>   | RAW38577.1     | 1 | MRMLFQVHLVFLVALFASCEAATPTKNQLVUSTNSTATAKALOKFFAEDAKONNNNGF     |
| <i>P. palmivora</i>  | Ppal15kDaA     | 1 | --MRMIQVVFMLLALFASCEATTTPTKHQLTISTINASATAKALEKFFTEDAKONNNNGF   |
| <i>P. megakarya</i>  | OWZ12091.1     | 1 | --MHIIQVVFVFLVLLFTSCESTTAPTKHOLTISTTNATATAKALEKFFTEDAKONKKNQY  |
| <i>P. parasitica</i> | XP_008909747.1 | 1 | --MLLIQVLLVFLVALFVSCAAA--TQNQLTIITTDSTATAKALOKFFTEDATONRNNGF   |
| <i>P. cactorum</i>   | RAW38578.1     | 1 | --MRMSQVLLVFLVTLVLSCEATA--AQNLTIATTNSTATAKALOKFFNEDSKONQNNQY   |

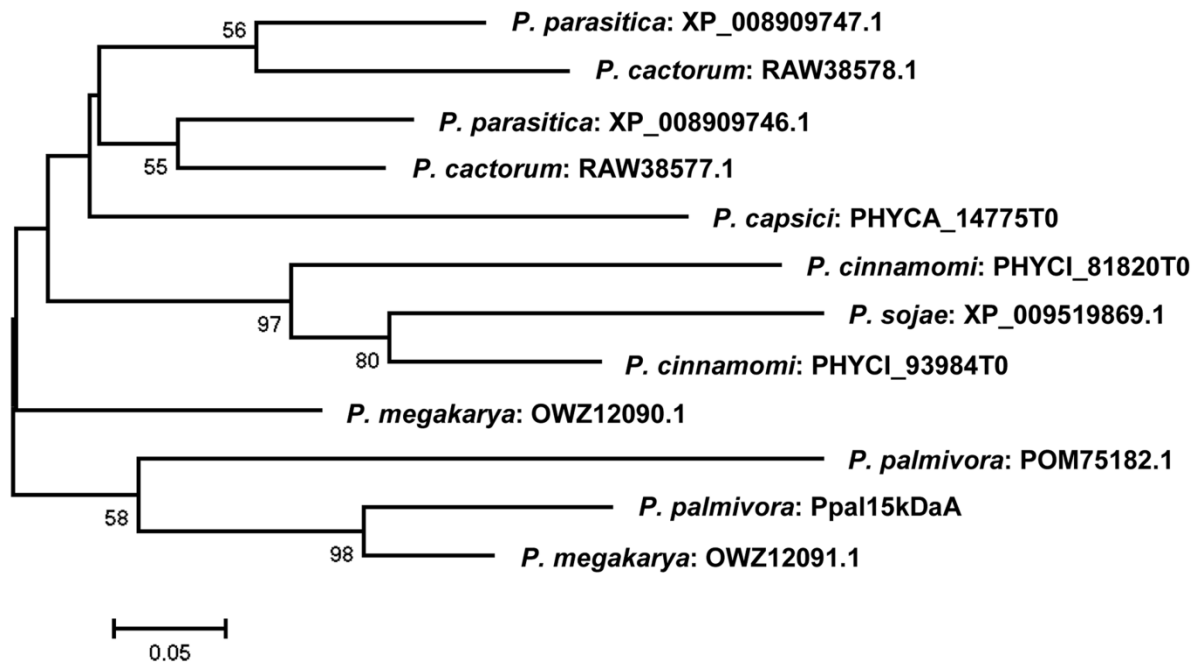
  

|                      |                |    |  |
|----------------------|----------------|----|--|
| <i>P. sojae</i>      | XP_009519869.1 | 60 | LMVLSGSAADDEERVSA--GAIAAAGEGARAGAGTTVVSSSTSGTTKTVTVTHYNNNGLWQR |
| <i>P. cinnamomi</i>  | PHYCI_93984T0  | 61 | LKVLTVTSAAEERVSA--GAIAAAGEGARAGAGTTVVSSSGSTQTVTVTHYNNNGLWQR    |
| <i>P. palmivora</i>  | POM75182.1     | 60 | DKVKRRPCL-D-----EACVGTTVVSSDTESGEEVTVTVTHYNNNGLWQR             |
| <i>P. capsici</i>    | PHYCA_14775T0  | 60 | LKVIITPSSS-DEERASA--GAIAAGEGARAGAGTTIVSSGPSSGETLFD-----        |
| <i>P. cinnamomi</i>  | PHYCI_81820T0  | 61 | LKMMNVSSA-DGEERGA--GAMASGCGPRVGAGTTVVSDGTGSSQTVTVTHYNNNGLWQR   |
| <i>P. megakarya</i>  | OWZ12090.1     | 58 | LKVVTLSSS-KEERV-----IASGCGPRAGAGTTVVSSDAGIGETVTVTVTHYNNNGLWQR  |
| <i>P. parasitica</i> | XP_008909746.1 | 61 | LKVVTLSSS-NEERASA--GAITSGCGPRAGAGTTVVANDASSGETVTVTVTHYNNNGLWQR |
| <i>P. cactorum</i>   | RAW38577.1     | 61 | LKVVTLSST-NEERASA--GAMTSGCGPRAGAGTTVVANDAPSGETVTVTVTHYNNNGLWQR |
| <i>P. palmivora</i>  | Ppal15kDaA     | 60 | LKVVTPASS-DEERAST--SVAAAGEGARAGTGTIVSSGTASGEMVTVTVTHYNNNGLWQR  |
| <i>P. megakarya</i>  | OWZ12091.1     | 60 | LKVVTPTSS-GEERAST--SAITAGEGARAGTGTIVVSSDTPSGEMVTVTVTHYNNNGLWQR |
| <i>P. parasitica</i> | XP_008909747.1 | 58 | LKVVTLPSS-EEERAST-SGAITAGEGARAAAGTTVVSSDTPSGETVTVTVTHYNNNGLWQR |
| <i>P. cactorum</i>   | RAW38578.1     | 58 | LKVVTLSSS-EEERASTSTGAITAGEGARVAAGTTVVANDPLGETVTVTVTHYNNNGLWQR  |

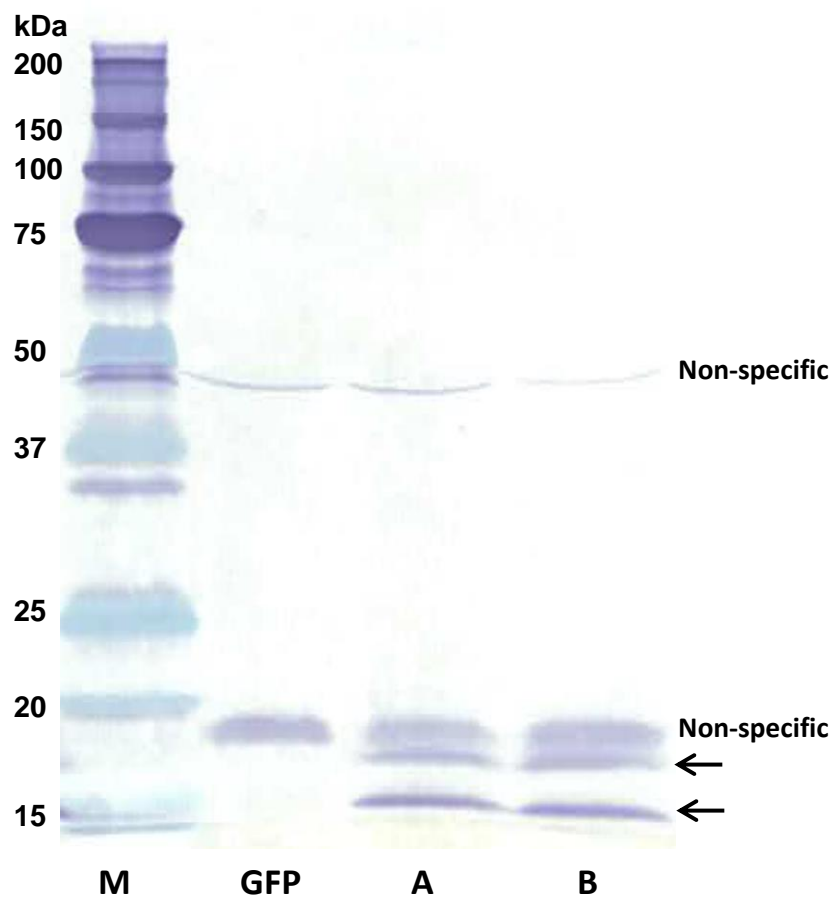
  

|                      |                |     |                            |
|----------------------|----------------|-----|----------------------------|
| <i>P. sojae</i>      | XP_009519869.1 | 118 | FORWWNSLFFARRRLRHASN-----  |
| <i>P. cinnamomi</i>  | PHYCI_93984T0  | 119 | FORWWNRLENDSTRRLRVAASGANN  |
| <i>P. palmivora</i>  | POM75182.1     | 102 | FORWWNRLLNGSTRRLRHPITGEQ-- |
| <i>P. capsici</i>    | PHYCA_14775T0  |     | -----                      |
| <i>P. cinnamomi</i>  | PHYCI_81820T0  | 118 | FORWWNRLEHRSSSSARRL-R----- |
| <i>P. megakarya</i>  | OWZ12090.1     | 111 | FORWWNRLENR-----           |
| <i>P. parasitica</i> | XP_008909746.1 | 118 | FLRWNNRLEFGSAANSTRRLRTDN-- |
| <i>P. cactorum</i>   | RAW38577.1     | 118 | FORWWNRLEKGSANSTRRLRTDN--  |
| <i>P. palmivora</i>  | Ppal15kDaA     | 117 | FORWWNSLPHSTSS--RLLRQE--   |
| <i>P. megakarya</i>  | OWZ12091.1     | 117 | FLRWNNRLEFYTSS--RLLRFS--   |
| <i>P. parasitica</i> | XP_008909747.1 | 116 | FLRWNNRLEHVSNN--RLLREGGKK  |
| <i>P. cactorum</i>   | RAW38578.1     | 117 | FLRWNNRLEHVSNN-STRLLREKV-- |

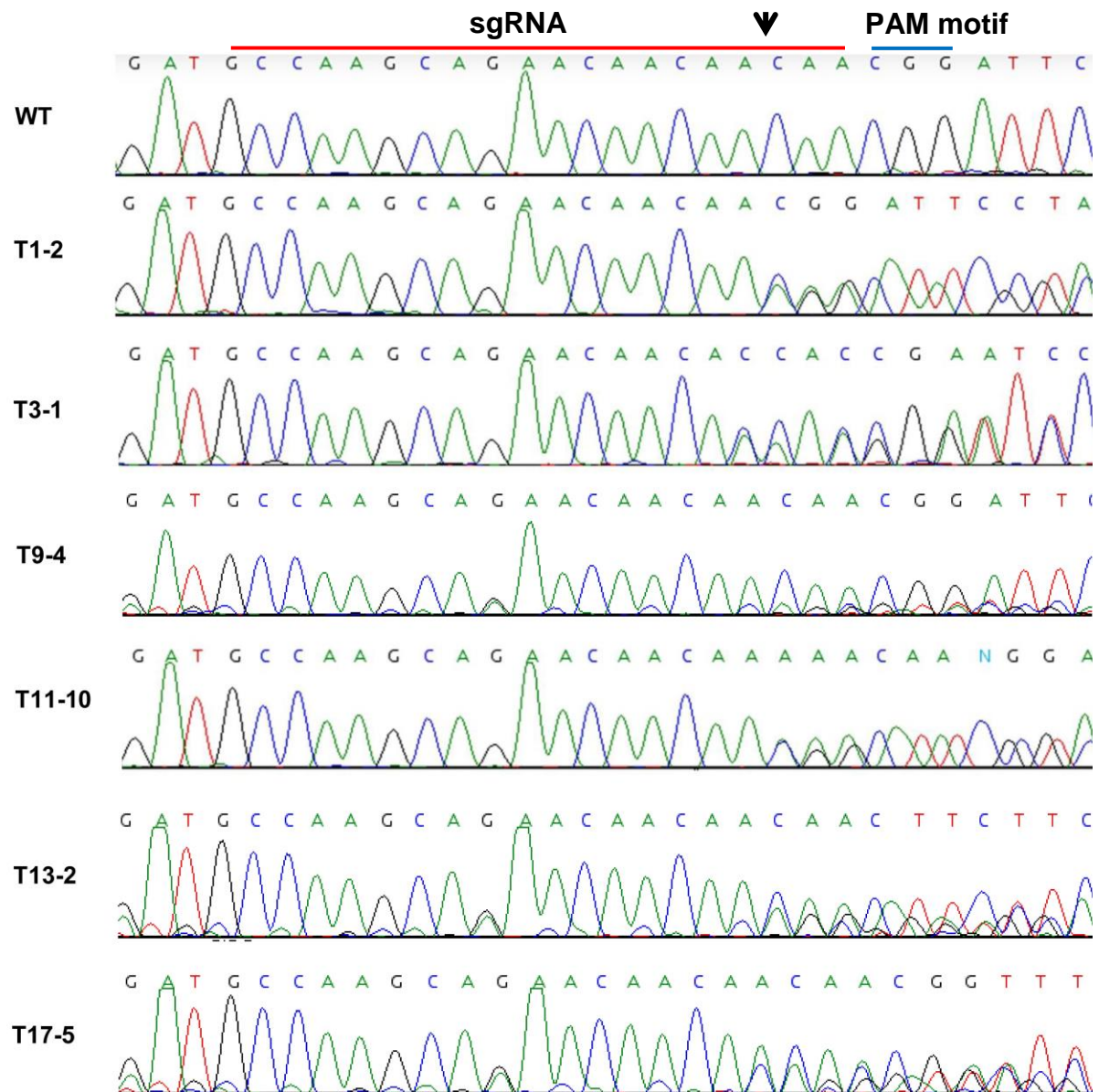
**Figure S2.** Phylogenetic analyses of Ppal15kDa and its homologs in *Phytophthora* spp. Construction of the phylogenetic tree was performed with the amino acid sequences using the neighbor-joining (NJ) method built in MEGA version 6.0. Bootstrap values were obtained with 1000 replicates and values higher than 50% are shown. The scale bar represents 0.05 amino acid substitutions per site.



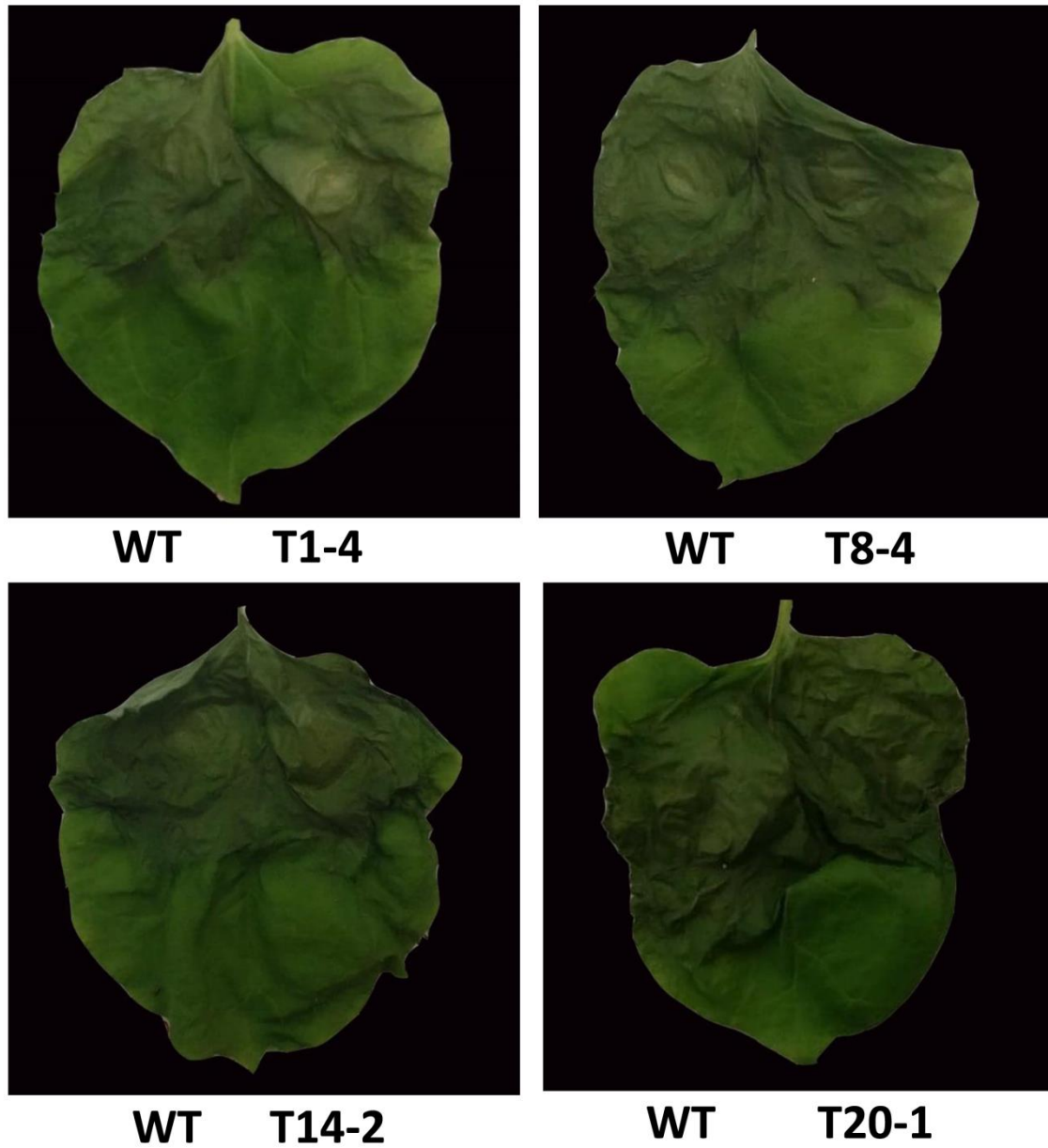
**Figure S3.** The full-length picture of Western blot analysis of total proteins extracted from infiltrated *N. benthamiana* leaves and subjected to SDS-PAGE followed by Western blot with HRP conjugated anti-His monoclonal antibody. Lane M represents the protein standard and lane GFP represents *N. benthamiana* leaves infiltrated with *A. tumefaciens* GV3101 carrying pJL-TRBO-G. Lane A and B represent *N. benthamiana* leaves infiltrated with *A. tumefaciens* GV3101 carrying pJL-TRBO-*Ppal15kDaA* and pJL-TRBO-*Ppal15kDaB*, respectively. The arrows indicate two forms of Ppal15kDa.



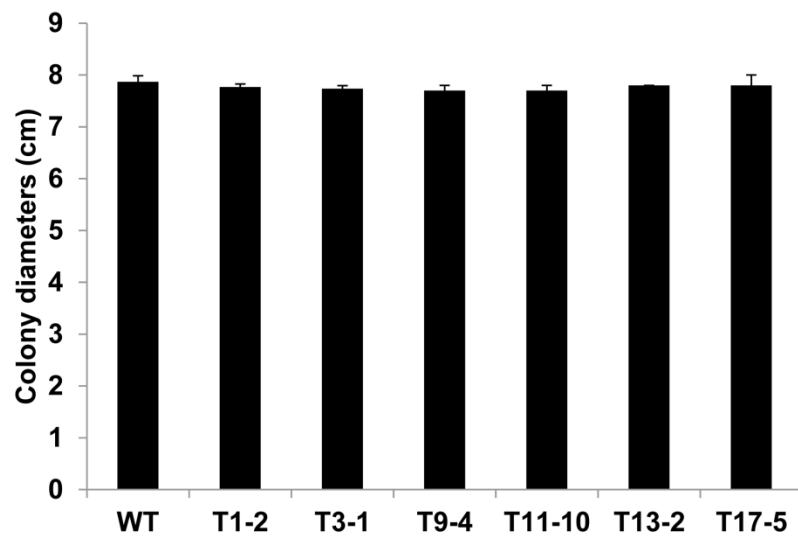
**Figure S4.** Chromatograms of the partial sequences of *Ppal15kDa* gene in mutants generated using CRISPR/Cas9 gene editing. The mutant lines exhibited mix peaks. The putative Cas9 cleavage site is shown by an arrow.



**Figure S5.** Infection assays of *Nicotiana benthamiana* leaves with *P. palmivora* wild-type (WT) strain and four representative lines that went through transformation but did not have mutations resulted from gene editing.



**Figure S6.** Mycelium growth of WT and mutants. WT and mutants were cultured on 10% unclarified V8 agar. The colony diameters were measured after 5 days.





**Figure S7.** Zoospore germination assay of *P. palmivora* wild-type (WT) strain and *Ppal15kDa* mutants. Zoospores were cultured on Plich agar for 4 hours and photographed under light microscope. Scale bars = 250  $\mu$ m.

